REMARKS

Upon entry of the foregoing amendment, claims 1-21, 52-72 and new claims 73-80 are pending in the application. Claims 22-51 were withdrawn by the Examiner under 37 C.F.R. § 1.142(b) as being directed to a non-elected invention, and have been canceled without prejudice to, or disclaimer of, the material recited therein. Claims 1-20 and 52-72 stand rejected under 35 U.S.C. § 103(a). Applicants note for the record that claim 21 was not addressed in the rejections made by the Examiner, but was not explicitly allowed. The amended claims are claims 1-4, 14, 19-21, 52-54, 56-65, and 67-72.

Independent claims 1, 52, and 63 are amended to describe that the vector of the invention uses a prokaryotic transposase, wherein the transposase gene is modified such that a plurality of the first ten codons of the transposase gene are individually modified from the wild-type sequence at the third base position of the codon to an adenine or thymine at the third base position of the codon, such that the modification does not change the amino acid encoded the modified codon. Independent claims 52 and 63 also recite that the modified transposase gene comprises an A or T at the third base position in each of codons 2-10. Claim 63 is further amended to describe that the vector may include a polyA sequence and two stop codons operatively linked to the modified transposase gene. Also, claims 2 and 53 are amended to recite an example sequence for a modified transposase gene used in a vector of the invention, and claim 3 is amended to recite the vector of SEQ ID NO: 1. Support for the amendment of claims 1-3, 52, and 63 is provided by the specification at page 58 and in the Appendix, showing the sequence of a vector of the invention SEQ ID NO: 1 where each of codons 2-10 of the transposase gene have an A or T at the third position of the codon, and claims 12 and 14, describing that the vector may comprise two stop codons and/or a poly A sequence operably-linked to the transposase. Claims 19, 20, 59, 60, and 68 and 69 describe that the egg directing sequence may be one of a ovalbumin, ovomucoid, or vitellogenin egg directing sequence and provide individual sequences for such elements. Other amendments to the claims correct the syntax or dependencies of the claims.

New claims 73-82 provide sequences for the components of the vectors, such as the gene of interest, promoters, enhancers, polyA sequences, and TAG sequences, as well as individual vectors of the invention. Support for the new claims is found in the

examples, describing specific vectors of the invention and in the Appendix, where the sequences for various vectors of the invention, and the individual elements that are utilized in each vector, are provided.

The specification is amended to remove the changes entered in Applicants' previous amendment and response and thus, is supported by the specification as originally filed. Accordingly, no new matter is added by the amendment of the claims or the specification.

The Rejection of Claims Under 35 U.S.C. § 103(a) Is Traversed Or Rendered Moot

A. Prima Facie Obviousness

Claims 1-6, 8-10, and 15-17

The Examiner rejected claims 1-6, 8-10 and 15-17 as being unpatentable over Cooper R., U.S. Patent No. 5,719,055 (hereinafter "Cooper"), Meiss (Biotechniques, 2000, 29(3): 476-480 (hereinafter "Meiss"), taken with Fischer et al., PNAS, 2001, 98(12), 6759-6764 (hereinafter "Fischer").

The Examiner asserted that Cooper teaches a vector comprising a gene encoding a transposase operably linked to a promoter, Mo transposon insertion sequences recognized by the transposase, where the vector includes an exogenous gene located between the transposon insertion sequences, and may also include an inducible promoter directing expression of the transposase gene. Office Action at 4. The Examiner stated that the claims require a modified transposase gene, wherein one to twenty codons, preferably the first ten, at a beginning of the transposase gene are modified by changing a nucleotide at a third base position of a codon to an adenine or thymine without changing the amino acid encoded by the codon, but asserted that such claim limitations embrace not only mutated but also wild-type transposase genes having an A or T in the third position of a codon at a beginning of a transposase gene. The Examiner referenced a wild-type Tn5 transposon gene sequence (Schulz et al., J. Mol. Biol., 1991, 221:65-80) as having codons in the beginning of the transposase gene with an A or T in the third position. Office Action at page 4.

The Examiner noted that Cooper differed from the claimed invention by not teaching a promoter comprising a modified Kozak sequence that comprises ACCATG or

a vector comprising more than one gene of interest operably linked to more than one promoter between the transposase insertion sequences, but stated:

> At the time the claimed invention was made inclusion of a Kozak sequence in an expression vector for optimal translation initiation of a gene in vertebrate cells was within the routine skill level of the ordinary artisan. . . . For example, Meiss et al. taught a vector for providing expression of a gene of interest in either prokaryotic or vertebrate cells. The [Meiss] vector comprised a CMV promoter in operable linkage with a Kozak sequence operably linked to a reporter gene and a sequence encoding a histidine tag. . . . Fischer taught cloning of Kozak sequence upstream of transposase gene and at 3' to the PRM1-NX promoter resulting in vector prm1-Tc1 . . . to express gene in mouse cells.

Office Action at page 5.

As amended, Applicants' claimed vector comprises: (a) a prokaryotic transposase gene operably linked to a first promoter, wherein the nucleic acid sequence 3' to the first promoter comprises the Kozak sequence as set forth in SEQ ID NO: 13, the Kozak sequence being positioned so as to include at least the first codon of the transposase gene, wherein the transposase gene is modified such that a plurality of the first ten codons of the transposase gene are individually modified from the wild-type sequence at the third base position of the codon to an adenine or thymine at the third base position of the codon, such that the modification does not change the amino acid encoded the modified codon; and (b) one or more genes of interest operably-linked to one or more additional promoters, wherein the one or more genes of interest and their operably-linked promoters are flanked by transposase insertion sequences recognized by a transposase encoded by the modified transposase gene.

The references fail to disclose elements of the claimed method

Applicants respectfully assert that the cited references do not, individually or in combination, teach or suggest a vector that uses a prokaryotic transposase gene (or any other prokaryotic gene) having a Kozak sequence as the first codon, and a transposase gene having a plurality of the first ten codons of the transposase gene modified from the wild-type sequence at the third base position of the codon to an adenine or thymine at

those positions where the change does not modify the amino acid encoded by the modified codon.

As noted by the Examiner, Cooper did not describe, teach or suggest a transposase vector utilizing a prokaryotic transposase in which a Kozak sequence is positioned so as to include at least the first codon of the prokaryotic transposase gene. Also, neither Meiss nor Fischer describe, teach or suggest the use of a Kozak sequence to increase translation of a prokaryotic gene. Instead, both Meiss and Fischer describe shuttle vectors having both prokaryotic and eukaryotic elements, but that use a Kozak sequence as part of a eukaryotic gene, to increase translation of eukaryotic gene sequences in a eukaryotic system.

Thus, Meiss is concerned with a vector that may be used *in vitro*, to allow for expression of eukaryotic proteins to be harvested from the *in vitro* culture. In contrast to Applicants, Meiss teaches using both prokaryotic (rbs) and a eukaryotic (Kozak) translation initiation sequences upstream of a eukaryotic gene to allow for expression of the eukaryotic gene (e.g., DFF40 or EGFP) in either a prokaryotic cell or a eukaryotic cell. Thus, Meiss does not describe, teach or suggest use of a Kozak sequence for expression of a prokaryotic protein, but is concerned with expression of eukaryotic proteins either in prokaryotic cells or eukaryotic cells. Meiss is specifically concerned with the construction of shuttle vectors, and there is no description, teaching or suggestion in Meiss of a construct that allows for the expression of a prokaryotic protein in eukaryotic cells.

Fischer describes a construct that includes a Kozak sequence preceding the open reading frame (ORF) of a eukaryotic transposase, the Tc1 transposase. There is no description in Fischer of a Kozak sequence being part of a prokaryotic gene sequence.

Also, as amended, Applicants' claimed vector includes a prokaryotic transposase wherein a plurality of the first ten codons of the transposase gene are modified from the wild-type sequence at the third base position of the codon to an adenine or thymine at those positions where the change does not modify the amino acid encoded by the codon. The Examiner has stated that the modified transposases of Applicants' vector embrace not only mutated but also wild-type transposase genes. However, the reference cited by the Examiner (i.e., Schultz et al., J. Mol. Biol., 1991, 221:65-80) does not describe a

transposase gene, wherein a plurality of the first ten codons of the transposase gene are modified from the wild-type sequence at the third base position of the codon to an adenine or thymine at those positions where the change does not modify the amino acid encoded by the codon, but in fact provides the sequence of the first four codons of a wild-type transposase. Nor does Schultz describe, teach or suggest a transposase gene, wherein the modified transposase gene comprises an A or T at the third position in each of codons 2-10 of the modified transposase gene, as provided by certain embodiments of Applicants' claimed vector.

Both explicit and inherent anticipation require that each element of the claim is necessarily present, or inherent, in the single anticipating reference. *See e.g., Glaxo Group Limited v. Apotex,* 376 F3d 1339 (Fed. Cir. 2004). If an element is missing, inherent anticipation is not found by showing probabilities or possibilities that the element is present in the prior art. *See e.g., Schering Corp. v. Geneva Pharm. Inc.,* 339 F.3d 1373, 1377 (Fed. Cir. 2003). As Schultz only describes a wild-type transposase sequence, Applicants respectfully assert that there is nothing in Schultz et al., that describes (or teaches or suggests) modifying a wild-type transposase gene in the manner described and claimed by Applicants.

There is no motivation to combine

Furthermore, even if the combination of the teachings of Cooper, Meiss and Fischer were to suggest Applicants' transposon-based vector constructs, which Applicants do not concede, there is absolutely no motivation to combine the teachings of these references to generate the claimed vectors of Applicants' invention.

As noted by the Examiner, the vectors of Cooper did not employ a Kozak sequence positioned so as to be included in at least the first codon of a prokaryotic transposase gene to promote translation of the prokaryotic transposae gene in eukaryotic cells. Nor did the vectors of Cooper employ a modified transposase gene as described and claimed by Applicants. Instead, Cooper teaches that a transposase-based vector may be designed without the use of a Kozak sequence to promote translation of the prokaryotic transposase gene in eukaryotic cells. Cooper also teaches that a transposase-based vector may be designed without using a transposase gene that is modified from the wild-type sequence to include either an A or a T at the wobble position in a plurality of

the first ten codons to promote strand dissociation of the transpose gene. Thus, one of skill in the art reading Cooper would not be motivated to incorporate a Kozak sequence in a transposon-based vector, or to include a modified transposase gene, as these modifications were not required for transformation of mammalian and/or fish cells as described in Cooper.

Also, Meiss is specifically concerned with the construction of shuttle vectors; there is no teaching in Meiss of a construct that employs a Kozak sequence to increase translation of a prokaryotic gene sequence in eukaryotic cells. Nor is there any description, teaching or suggestion in Meiss of a prokaryotic gene that is modified at a plurality of codons at the N-terminus to have the wobble position of the codon changed to an adenine or thymine at those positions where the change does not modify the amino acid encoded by the codon to thereby facilitate strand dissociation. Thus, there is no motivation to combine Cooper with Meiss to arrive at the transposase-based vectors described and claimed by Applicants.

Similarly, there is no motivation provided by Fischer to use a Kozak sequence as part of a prokaryotic transposase gene, or a prokaryotic transposase gene modified at a plurality of codons at the N-terminus to have the third base position of the codon changed to an adenine or thymine at those positions where the change does not modify the amino acid encoded by the codon, as Fischer simply describes a eukaryotic transposase (Tc1/mariner) that includes a Kozak sequence. Thus, one reading these references would not be motivated to incorporate a Kozak sequence in a prokaryotic gene, or to modify a transposase gene sequence so as to have the third base position of the codon changed to an adenine or thymine at those positions where the change does not modify the amino acid encoded by the codon, as is described and claimed by Applicants.

For at least the above-reasons, Applicants respectfully assert that, without the hindsight gained by the disclosure of the instant application, the references of Cooper, Meiss, and Fischer alone, or in combination, do not describe, teach or suggest including a Kozak sequence as the N-terminus of a prokaryotic transposase, and/or modifying the transposase gene at the N-terminus to have the third base position of the codon changed to an adenine or thymine at those positions where the change does not modify the amino

acid encoded by the codon to thereby increase expression of a prokaryotic transposase in eukaryotic cells.

For at least these reasons, Applicants respectfully assert that claims 1-6, 8-10 and 15-17 are not *prima facie* obvious under 35 U.S.C. § 103, and respectfully request that the rejection be withdrawn.

B. Claims 1-11, 15-20, 52-53, and 57-62

The Examiner rejected claims 1-11, 15-20, 52-53, and 57-62 as being unpatentable over Cooper, Meiss, and Fischer, in view of Hackett et al., U.S. Patent No., 6,489,458 (hereinafter "Hackett"). Thus, the Examiner stated:

> [N]one of the references [Cooper, Meiss and Fischer] explicitly teaches advantage of using ovalbumin or other inducible promoters. Hackett et al teach introducing nucleic acid encoding the SB transposase gene operably linked to a promoter. . . . Hackett et al disclose variety of promoters that could be used including constitutive promoters, tissue-specific promoters, and inducible promoter . . . In addition, Hackett teaches protein can be produced in quantity in milk, urine, blood or eggs by using promoters known for expression in milk, urine, blood or eggs such as ovalbumin promoter.

Office Action at page 7 (emphasis in original).

Applicants respectfully assert that Hackett does not remedy the deficiencies of Cooper, Meiss and Fischer. Hackett describes using the Sleeping Beauty family of eukaryotic transposases as a system for introducing nucleic acid into the genome of a vetebrate cell. Hackett describes a series of experiments by which a transposase gene is reconstructed from inactive fragments (see Hackett at Example 1). Notably, in designing the SB transposase system, Hackett does not describe, teach or suggest using a prokaryotic transposase for expression of a gene of interest in a eukaryotic system. In fact, Hackett specifically teaches that most transposases are species-specific, and that only the (eukaryotic) Tc1/mariner superfamily of transposases would be expected to be useful for cross-species transposition. Hackett at col. 2, lines 6-14. Nor does Hackett describe, teach or suggest a vector including a prokaryotic transposase having a Kozak sequence being positioned so as to include at least the first codon of the transposase gene, or that such a construct would be beneficial and provide increased expression of the

transposase and/or integration of the construct in a eukaryotic genome. There is also no mention in Hackett of modifying a prokaryotic transposase gene at the 5' end of the gene to have the third base position of the codon changed to an adenine or thymine at those positions where the change does not modify the amino acid encoded by the codon for increased strand dissociation and expression of the prokaryotic transposase in eukaryotic cells.

Also, Hackett only provides a generic description that promoters and other regulatory elements may be used in transposon-based vectors. Hackett does not, however, describe, teach, or suggest the specific nucleotide sequences of constitutive and inducible promoters, enhancer elements, and signal sequences that may be used for the transposase gene and the gene of interest, to allow for the targeted insertion and expression of a gene in particular cells as is provided by Applicants' claimed invention. In addition to the increased insertion frequencies provided by Applicants' vectors (see below), Applicants' claimed vectors allow for versatility in selection of the gene of interest, as well as the tissue type in which the gene is expressed. For example, Applicants' vectors provide for using constitutive promoters to express the transposase (and thus insert the gene of interest) in all types of cells, in conjunction with inducible promoters (e.g., ovalbumin promoter and vitellogenin promoters) that may be used to express the gene(s) of interest in specific cells or tissue types.

Thus, Hackett does not remedy the deficiencies of Cooper, Meiss and Fischer in that Hackett does not describe teach or suggest a transposon-based vector that includes a Kozak sequence at the 5' end of a prokaryotic transposase gene, and/or a modified transposase as is described and claimed by Applicants. Also, the generalized description of promoters provided by Hackett does not describe, teach or suggest the specific sequences provided by Applicants' claimed vectors.

For at least these reasons, Applicants respectfully assert that claims 1-11, 15-20, 52-53, and 57-62 are not *prima facie* obvious under 35 U.S.C. § 103, and respectfully request that the rejection be withdrawn.

C. Claims 1-20 and 52-72

The Examiner rejected claims 1-20 and 52-72 as being unpatentable over Cooper, Meiss, Fischer, and Hackett in view of Wallace and Sanders (Biology: The Science of Life, 1986 (hereinafter "Wallace et al.). Thus, the Examiner stated:

> Prior to filing of this application, Wallace et al., teach three stop codons UAA, UAG, and UGA that are used as stop codon. It is noted that Wallace et al also disclose double stop codon such as UAA-UAG to ensure message to ribosome (pp 235, col. 2, see section polypeptide chain termination). . . . One of ordinary skill in the art would have been motivated to include two-stop codon[s] to ensure proper termination of transposase synthesis and would have also included poly A as a[n] obvious modification for expression in mammalian system.

Office Action at pages 8-9.

Applicants respectfully assert that Wallace does not correct the deficiencies of the references (Cooper, Meiss, Fischer, and Hackett) discussed above. Further, as amended, Applicants' vectors comprise a prokaryotic transposase gene operationally linked to a polyA sequence and/or two stop codons (claims 12, 14, 63). Applicants respectfully assert that there is no description, teaching or suggestion provided by Wallace or other teachings in the art to include a polyA sequence that is operably-linked to a prokaryotic gene. Most prokaryotic genes do not include polyA sequences, and for the few prokaryotic genes that do include polyA sequences, the function of the polyA sequences is different than in eukaryotes. For these reasons, there was no expectation of success that a polyA sequence could be operably linked to a prokaryotic transposase gene and correctly positioned relative to the transposase gene and its associated stop codons. It was known in the art that improper positioning of the polyA sequence relative to a gene and its associated stop codons can result in a decrease in mRNA stability, resulting in less protein being produced. Further, a review of the literature indicates that it has not been possible to predict what sequence elements downstream of the stop codon, other than the polyA, are involved in stability, again indicating that there was no inherent expectation of success that it would be possible to operably link a polyA sequence to a prokaryotic gene. Thus, one would not be motivated to rely on the use of polyA sequences to increase

expression of a prokaryotic gene as it was not clear as to whether such an approach would be successful.

For at least these reasons, Applicants respectfully assert that claims 1-20 and 52-72 are not *prima facie* obvious under 35 U.S.C. § 103, and respectfully request that the rejection be withdrawn.

B. Secondary Considerations

Additionally, secondary considerations render the claimed invention as not obvious over the cited references. The vectors of the present invention provide constructs that allow for the unexpected result of greatly improved expression of the transposase protein, thereby resulting in a significant increase in insertion frequencies. Applicants note that the vectors of the invention are far more efficient than vectors used in the prior art. Thus, as noted in the specification at page 13, lines 15-23, the vectors of the present invention produce integration frequencies an order of magnitude greater than other vectors commonly used at the time of the invention and almost a 2-fold increase in insertion frequency over the vectors of Cooper.

Additionally, Applicants submit herewith a Declaration Under C.F.R. § 1.132 that using a vector of the invention (pTnMod as described in Example 1 of the specification) with a monoclonal antibody as the gene of interest appeared to exhibit very high efficiency in transfecting both the livers and ovaries of female quails that were injected via a cardiac route. Applicants are not aware of any other vectors that approach this level of efficiency. The vectors used include a modified transposase having a Kozak sequence positioned to include the first codon, a plurality of the first ten codons of the transposase gene individually modified from the wild-type sequence at the third base position of the codon to an adenine or thymine at the third base position of the codon, such that codons 2-10 have an A or a T at the third (wobble) position of the codon, as well as two stop codons and a poly A sequence operably linked to the transposase.

Thus, Applicants respectfully assert that claims 1-20 and 52-72 are not obvious under 35 U.S.C. § 103, and respectfully request that the rejection be withdrawn.

CONCLUSION

In view of the foregoing amendment and remarks, each of the claims remaining in the application is in condition for immediate allowance. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the outstanding rejections. The Examiner is respectfully invited to telephone the undersigned at (336) 747-7541 to discuss any questions relating to the application.

Respectfully submitted,

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